

Supplemental Materials and Methods

Generation of LSL.eGFP.miR-26a transgenic mice

A 333bp fragment containing the pre-miR-26a-2 hairpin was amplified by PCR from human gDNA using primers containing *NotI* sites and then cloned into pEGFP-N1 (Clontech) at the *NotI* site downstream of EGFP to yield eGFP.miR-26a. A fragment of the LSL was excised from the previously described LSL-KrasG12D targeting vector (Jackson et al., 2001) by *RsrII* digestion followed by a partial digestion with *EcoRI* to yield a 5318bp fragment containing Stop-Lox. This was then cloned into the *EcoRI* site upstream of eGFP in eGFP.miR-26a. The upstream loxP site was created by amplification of self-complementary oligonucleotides to create a loxP sequence flanked by *EcoRI*, *HindIII*, and *SrgA1* sites. After cleavage with *HindIII* and *SgrA1*, the loxP site was inserted upstream of Stop-Lox to generate LSL.eGFP.miR-26a. A partially digested *EcoRI* fragment containing LSL.eGFP.miR-26a was inserted into the pBS31' vector (Thermo) downstream of the tetO minimal promoter to generate the final targeting vector, which was used as previously described to insert the transgene downstream of the *Col1A1* locus in KH2 embryonic stem cells (Beard et al. 2006). Primer sequences are provided in Supplemental Table S2.

BrdU pulse labeling

Dox treatment was initiated 7 days prior to intraperitoneal injection of 100 µL of 20 mg/mL BrdU (Sigma). Mice were sacrificed at various time-points after injection and intestines were fixed and immunostained with anti-BrdU.

Histology, immunohistochemistry, and immunofluorescence

Intestinal tissues were harvested for histologic analysis as previously described (Chivukula et al. 2014). The following primary antibodies and dilutions were used: anti-BrdU (Developmental Studies Hybridoma Bank, #G3G4; 1:200), anti-E-cadherin Alexa Flour 488-conjugated antibody (Cell Signaling, #3199, 1:100 dilution), anti-phosphorylated histone 3 (Millipore #06-570, 1:100 dilution). VECTASTAIN Elite ABC Reagent with 3'-3' diaminobenzidine substrate (Vector Labs) or fluorophore conjugated secondary antibodies (Invitrogen; 1:200) were used for signal detection. GFP immunohistochemistry was carried out using the TSA plus Biotin Kit (Perkin Elmer) with anti-GFP (Invitrogen, #A11122, 1:200). Alkaline phosphatase staining was performed by applying NBT/BCIP (Roche) at a final concentration of 0.4mg/ml NBT, 0.19mg/ml BCIP, 100mM Tris pH 9.5, 50mM MgSO₄ for 2 hours at room temperature. Images were acquired on a Zeiss AxioObserver Z1 microscope using the MosaiX/Stitching function of AxioVision, which produces a composite image of multiple adjacent fields.

RNA isolation, quantitative RT-PCR, and microarray analysis

Total RNA was isolated using the miRNeasy Mini Kit (Qiagen) according to the manufacturer's protocol. Pre-designed Taqman primers and probes (Life Technologies) were used according to the manufacturer's protocol to measure the levels of mature miRNAs, U6 snRNA, and *Lgr5* mRNA. All other mRNAs were reverse transcribed using Quantitect (Qiagen) and abundance was assayed using Power SYBR Green Mastermix (Life Technologies). Primer sequences are provided in Supplemental Table S2. Microarray experiments were performed on triplicate samples of purified epithelial cells

from dox-treated *M2rtTA* and *M2rtTA; eGFP.miR-26a* mice using the MouseWG-6 V5 BeadChip (Illumina) at the UTSW Microarray Core. Results were analyzed using GeneSpring software (Agilent).

Western blots

Tissue extracts were prepared by homogenization in RIPA buffer (50mM Tris pH 8.0, 150mM NaCl, 1% NP-40, 0.5% Sodium deoxycholate, 0.1% SDS). Blots were probed with anti-PTEN (1:2000; Cell signaling, #9188) and anti-GAPDH (1:4000; Cell Signaling, #2118) antibodies. Films were scanned and signals were quantified using Quantity One 1-D Analysis Software (Bio-Rad).

Luciferase reporter assays

Reporter constructs were created by cloning the predicted miR-26 binding sites and ~225 bp of the flanking the 3' UTR sequence into the XbaI site of pGL3-control (Promega). Mutagenesis was carried out by re-amplifying each fragment with primers that included a mutated binding site and re-introducing the mutated site into the reporter plasmids using the In-fusion Cloning Kit (Clontech). Primer sequences are provided in Supplemental Table S2. 16 hours after plating 2.5×10^5 HCT116 cells in triplicate wells of a 24-well plate, Lipofectamine 2000 (Life Technologies) was used to transfect cells with 100 ng of the indicated reporter construct, 5 ng of phRL-SV40 (Promega), and 25 nM non-target or miR-26a mimics (Dharmacon). After 24 hours, firefly and renilla luciferase activity was assayed using the Dual Luciferase Reporter Assay System

(Promega) according to the manufacturer's protocol. Renilla luciferase activity was used to normalize firefly luciferase activity for each well.

Supplemental Reference

Jackson EL, Willis N, Mercer K, Bronson RT, Crowley D, Montoya R, Jacks T, Tuveson DA. 2001. Analysis of lung tumor initiation and progression using conditional expression of oncogenic K-ras. *Genes Dev* **15**:3243-3248.